AD-A162 953 DEPENDENCE OF ENDOTHELIAL CELL PROSTACYCLIN SYNTHESIS ON CELL SHAPE AND F (U) ARMY RESEARCH INST OF ENVIRONMENTAL MEDICINE NATICK MA D A DUBOSE ET AL 1/1

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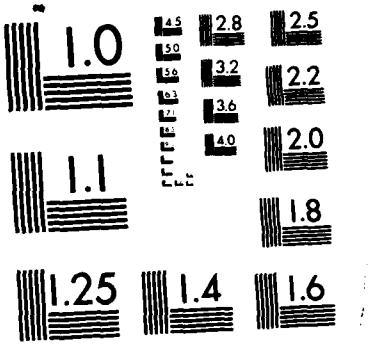

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DEPENDENCE OF ENDOTHELIAL CELL PROSTACYCLIN SYNTHESIS ON CELL SHAPE AND F-ACTIN CYTOSKELETAL ARRANGEMENT

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Abstract

Alterations in endothelial cell shape, as measured by the planar surface area encompassed by the cell perimeter adhering to the substrate, were associated with modulation of basal prostacyclin synthesis. Cells with small surface area generated the highest levels of prostacyclin and had both a diffuse arrangement of F-actin and an increased binding capacity for rhodamine-phalloidin. Large cells with many actin cables and a significantly reduced phalloidin binding capacity produced significantly smaller quantities of prostacyclin. These findings suggest a cytoskeletal role in the regulation of prostacyclin production and more specifically, that changes in F-actin arrangement modulate basal endoperoxide metabolism.

Though numerous factors have been identified which modulate prostacyclin (PGI_2) synthesis (1), there is little information on cellular mechanisms whereby endothelial cells regulate their arachidonic acid metabolism. Since selective modulation of endoperoxide metabolism between platelets and endothelial cells is a major goal of antithrombotic therapy (2), an understanding of the cellular mechanisms by which phospholipids are converted to prostaglandins may provide insight to novel pharmacological approaches to change production in one cell type without altering release in another. Furthermore, because PGI_2 promotes actin stress fiber assembly, which is believed to be important to endothelial barrier function (3), control of its synthesis has ramifications beyond those associated with thrombosis. This report establishes a close correlation between basal PGI_2 synthesis and endothelial cell shape. Since changes in cell morphology imply alterations in the cytoskeletal framework, such a finding led to the exploration of the cytoskeleton as an important component in the mechanism whereby endothelial cells modulate their PGI_2 production.

The rationale for assuming a connection between cell shape and metabolism comes from in vitro findings which demonstrate that a flattened endothelial cell, associated with sparse culture, produces less PGI_2 than its more rounded counterpart as seen in a confluent, contact-inhibited culture (4). Folkman and Moscona found that cell to cell contact was not necessarily the most salient feature associated with modulation of DNA synthesis (5). They established the concept that the impact of contact

inhibition on metabolism was attributable to endothelial cells assuming a more spheroidal conformation as a result of cell packing. In their study, poly 2-hydroxethyl methacrylate (polyHEMA) was used to alter the adhesive capacity of tissue culture plastic. This reduced cell spreading and resulted in sparse cultures of cell shapes similar to that noted at confluency. In these cultures lacking cell to cell contacts, DNA synthesis was not significantly different from confluent, contact-inhibited cultures. Using a similar experimental approach, we have quantitated basal PGI_2 synthesis of cultures with a range of cell shapes obtained by seeding aortic endothelial cells on substrates of various adhesive capacities (6).

Samples for radioimmunoassay for the stable metabolite 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) (7) were collected from 24 h cultures, after these cultures had been washed twice and incubated for 30 min at 37°C in the presence of unsupplemented medium (8). Following sample collection, cultures were either fixed with 3.7% formalin or treated with 1.0 N NaOH for protein determination (9). Fixed cultures were stained with rhodamine-phalloidin (0.165 μm; Molecular Probes, Junction City, OR) following previously described procedures for fluorescence microscopy (10). From photographs of the rhodamine fluorescence, differences in cell shape were quantitated using a digital image analyzer to measure the planar surface area encompassed by the cell perimeter adhering to the substrate (cell perimeter surface area, CPSA). Since phalloidin binds specifically to F-actin (11), the distribution of this cytoskeletal constituent could be categorized by a convention similar to that

established by Verderame et al. (12), whereby cells were given a number (Verderame value) between 1 and 4, based on the degree to which actin cables could be noted (Figure 1). From these data, correlation and regression analyses between PGI_2 synthesis and either endothelial cell shape or F-actin distribution were made (Figure 2). To illustrate further the significance of F-actin arrangement in the regulation of arachidonic acid metabolism, a comparison of PGI_2 synthesis with CPSA, Verderame value and the rhodamine-phalloidin binding capacity of cells on substrates with extremes of cellular adhesivity was conducted (Table 1).

Figure 1 illustrates the alterations in cell shape and F-actin distribution that could be obtained by culturing bovine aortic endothelial cells on substrates of various adhesive capacities. The presence of visible actin cables appears to diminish as cells assume a more rounded and less flattened morphology. Hence, as Verderame value increased, the presence of perceptible actin cables and CPSA decreased.

Correlation analysis indicated that CPSA and PGI_2 synthesis covaried in a negative manner, whereas Verderame value and synthesis demonstrated a positive covariance (Figure 2). However, of greater significance, regression analysis illustrated that differences in basal PGI_2 metabolism was strongly dependent on modulation of CPSA and Verderame value, for both porcine and bovine aortic endothelial cells (Figure 2). As such, PGI_2 synthesis significantly increased when vascular endothelial cells were more rounded (reduced CPSA) and actin cables were less distinct (high Verderame value).

A comparison of the amount of rhodamine-phalloidin bound by cultures generating significantly different ($p < 0.05$) levels of PGI_2 revealed that the absence of actin cables and elevated PGI_2 production was associated with a significantly increased rhodamine-phalloidin binding capacity (Table 1). Though this might be interpreted to mean such cells have increased levels of F-actin, this finding is more likely a measure of the significant difference in the F-actin distribution. As noted by Heacock et al., alterations in cell morphology are not necessarily associated with a change in concentration of the G and F actin pools (13). Thus, the increased rhodamine-phalloidin binding by cells of high Verderame value (Figure 1d) may reflect the existence of F-actin as many fine indistinguishable fibers. These cells would then have an increased free fiber surface area for binding increased amounts of phalloidin. Such a condition would be absent in cells of lower Verderame value (Figure 1a) since the actin cables would have an overall reduced surface area for binding, due to the bundling of individual actin fibers to form a visible cable. Therefore, the difference in rhodamine-phalloidin binding illustrates the significant difference in F-actin arrangement (actin fibers vs. actin cables) between cultures of significantly different Verderame values and PGI_2 production capacities. This finding corroborates the actin distribution data determined by Verderame value (Figure 2) which demonstrated the dependence of enhanced PGI_2 production on a diffuse arrangement of F-actin.

Germane to these findings are the studies demonstrating the affinity of glycolytic enzymes for actin and actin-containing structures (14).

From this work has evolved the principle of metabolic compartmentation through the binding of key enzymes and metabolites to components of the cytoskeleton. Furthermore, other studies have shown that such binding of certain glycolytic enzymes alters their kinetic characteristics (15). Therefore, the cytoskeleton appears to have some control over both the freedom and activity of enzymes within the cell. In a similar fashion, enzymes of the arachidonic acid cascade may also be under the influence of the cytoskeleton. Thus, changes in cell shape, resulting in a shift in the arrangement of F-actin within the cell, may adjust the activity of important enzymes in this pathway, "fine tuning" the basal metabolic rate of the endoperoxides.

Our results indicated that basal PGI_2 release was significantly dependent on the arrangement of the cytoskeletal constituent F-actin. Endothelial cells of reduced CPSA with few discernible actin cables, as revealed by rhodamine-phalloidin fluorescence, generated significantly increased levels of PGI_2. Cultures with F-actin arranged as many visible cables were associated with a reduced capacity to synthesize PGI_2. These findings lead to the conclusion that the cytoskeleton perhaps plays a fundamental role in the mechanism controlling PGI_2 synthesis by endothelial cells. Understanding the specifics whereby cytoskeletal constituents contribute to the regulation of endothelial cell PGI_2 synthesis may prove important in the quest of selective modulation of endoperoxide metabolism within the vascular system.

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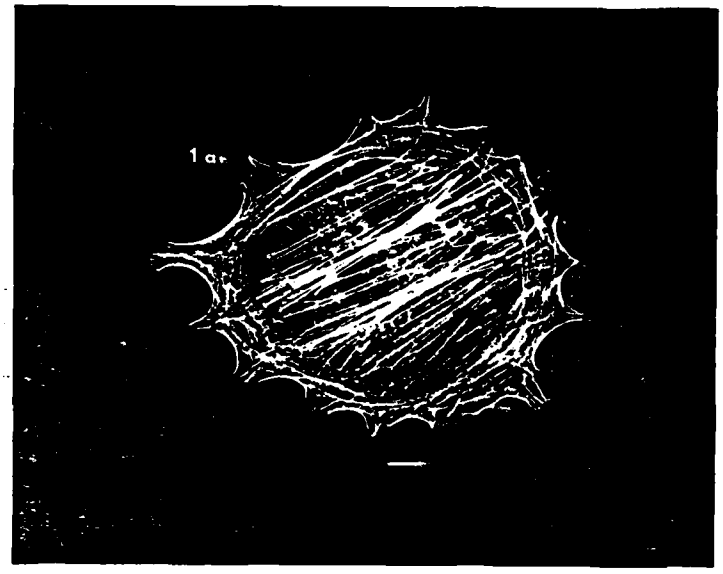
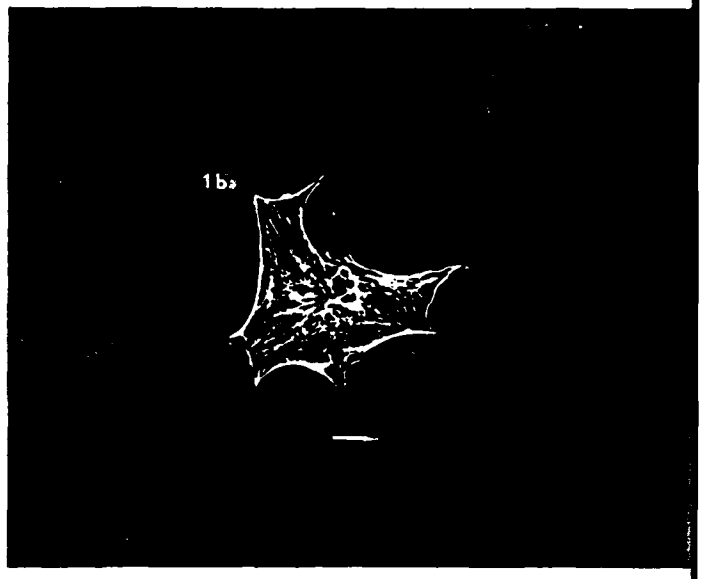
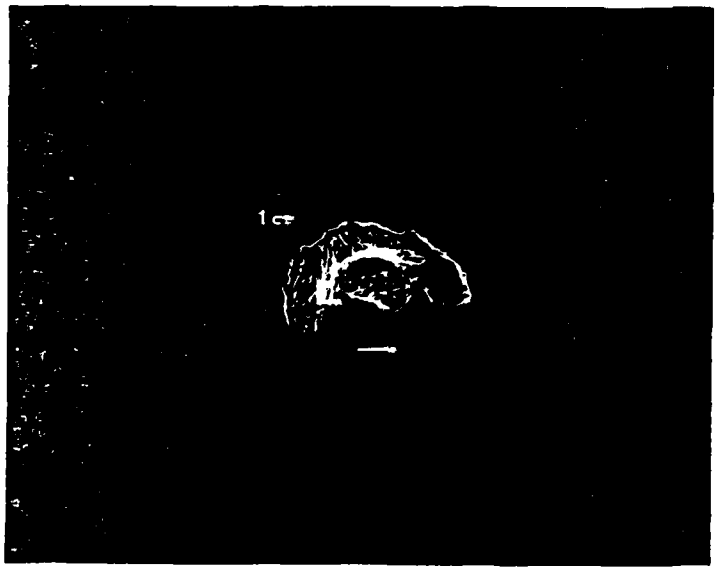
Figure 1. Bovine aortic endothelial cells cultured on substrates of various adhesive capacities (6), demonstrating the four types of F-actin distribution as described by Verderame (12), following treatment with rhodamine-phalloidin. (A) Type 1; cell contains many thick actin cables (tissue culture plastic). (B) Type 2; cell has both thick and fine actin cables (polyHEMA; 1.09 $\mu g/cm^2$). (C) Type 3; cell has only fine actin cables (polyHEMA; 4.37 $\mu g/cm^2$). (D) Type 4; cell has a diffuse arrangement of F-actin with few, if any, discernible actin cables (bacteriological plastic). Bar equals 10μ.







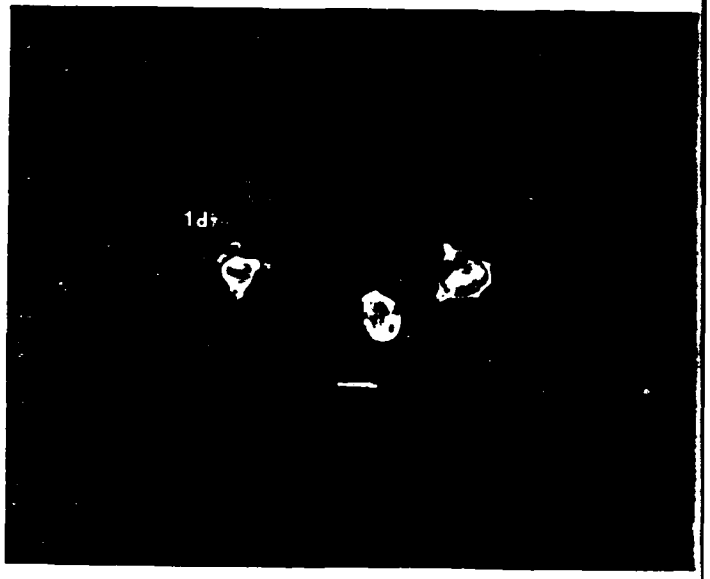
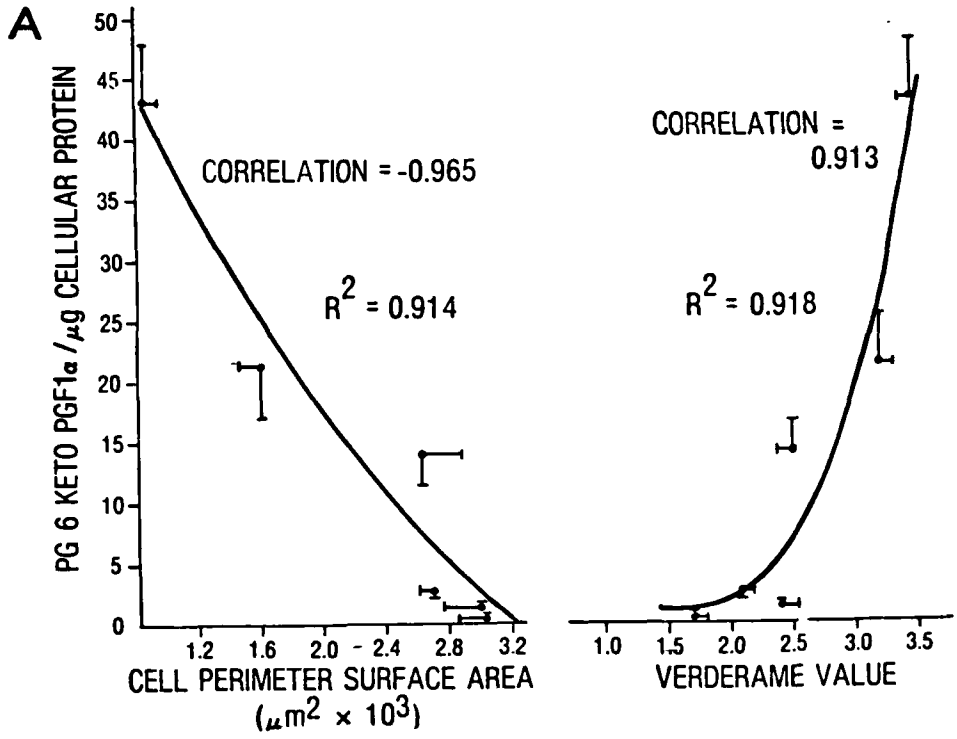


Figure 2. Correlation and regression analysis between PGI_2 production, and either cell perimeter surface area (CPSA) or Verderame value for (A) porcine aortic endothelial cells, and (B) bovine aortic endothelial cells cultured on substrates of various adhesive capacities (6). Each point for PGI_2 production represents a mean value determined from eight separate cultures. Both CPSA and Verderame value for each culture was determined by the measurement of at least 50 cells. From these determinations, a mean CPSA and Verderame value was calculated. Bars associated with each point reflect standard error. Categorizing cells for Verderame value was performed by two individuals unaware of the experimental parameters. Three replications of these experiments concluded with similar findings.


A
PG 6 KETO PGF1α /μg CELLULAR PROTEIN
50
45
40
35
30
25
20
15
10
5
0
CORRELATION = -0.965
R2 = 0.914
1.2
1.6
2.0
2.4
2.8
3.2
CELL PERIMETER SURFACE AREA
(μm2 × 10³)
CORRELATION =
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R2 = 0.918
1.0
1.5
2.0
2.5
3.0
3.5
VERDERAME VALUE

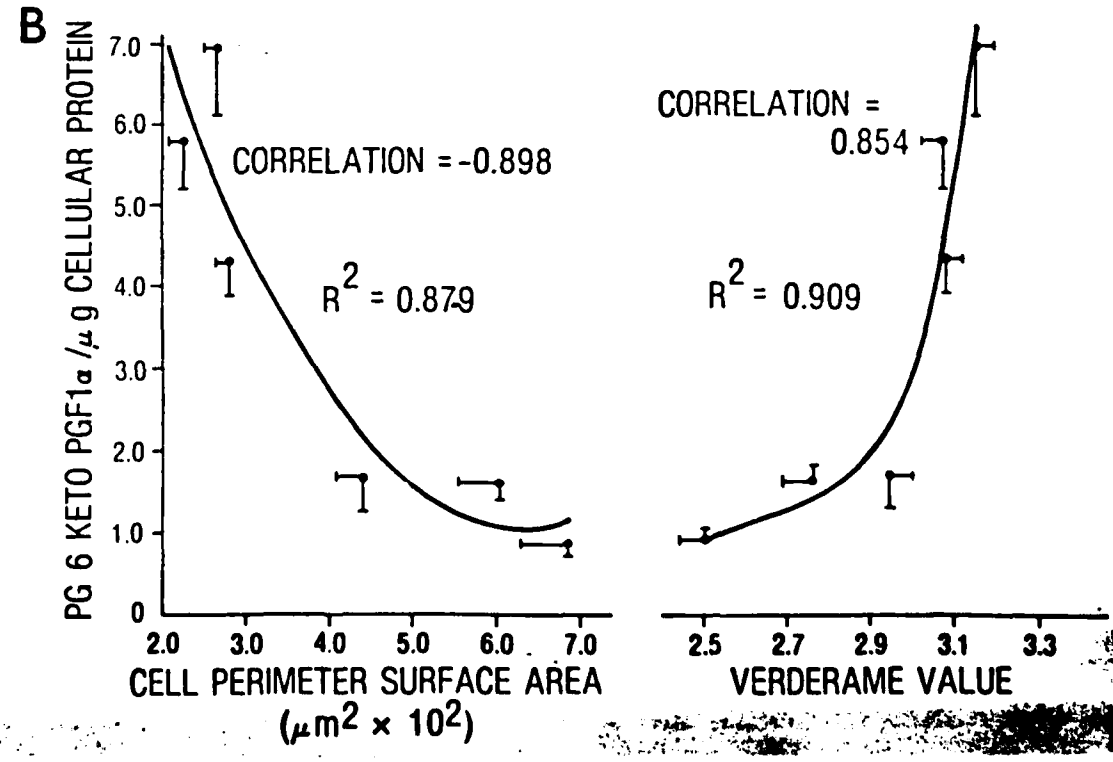
B
PG 6 KETO PGF1α /μ g CELLULAR PROTEIN
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
CORRELATION = -0.898
R2 = 0.879
2.0
3.0
4.0
5.0
6.0
7.0
CELL PERIMETER SURFACE AREA
(μm2 × 10²)
CORRELATION =
0.854
R2 = 0.909
2.5
2.7
2.9
3.1
3.3
VERDERAME VALUE

Table 1. Comparison of PGI_2 production with cell perimeter surface area (CPSA), Verderame value and rhodamine-phalloidin (R-P) binding capacity[#] by bovine aortic endothelial cells seeded on tissue culture or bacteriological plastic.

	Plastic substrate type	
	Tissue culture	Bacteriological
6 keto $PGF_{1\alpha}$ (pg/ug cell protein)	1.33 ± 0.16[A]	6.14* ± 0.51
CPSA (μm^2)	1399.3 ± 148.3	225.2* ± 13.5
Verderame Value	2.15 ± 0.06	3.08* ± 0.05
R-P (nmoles/ug cell protein)	1.88 ± 0.14	6.49* ± 0.69

[A] values are means ± standard error

[#] Cultures were fixed (3.7% formalin, 24 hrs), stained with rhodamine-phalloidin (0.165 µM) for 10 min and washed in saline to remove any unbound material. Cells were then removed with the aid of a rubber policeman and sonicated (1 min) to achieve a suspension. Fluorescence was determined with a spectrophotoflurometer using an excitation wavelength of 525 and an emission wavelength of 582. Relative light intensity emitted by the samples was then compared to a standard curve for relative light intensity generated for concentrations of rhodamine-phalloidin ranging between 0.165 µM to 0.004 µM. Increasing the rhodamine-phalloidin concentration twofold and the staining period to 1 h at 37°C did not alter the findings. Furthermore, results were not

altered by the use of DNase buffer (13), prior to staining, to prevent any possible binding of phalloidin to oligermers of G-actin.

* Significantly different ($p < 0.05$).

DISCLAIMER

The investigator adhered to the "Guide for Laboratory Animal Facilities and Care as promulgated by the Committee on the Guide for Laoratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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